Zurich Open Repository and Archive
University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2013

Evaluation of eco-toxicological effects of the parasiticide moxidectin in comparison to ivermectin in 11 species of dung flies

Blanckenhorn, Wolf U ; Puniamoorthy, Nalini ; Scheffczyk, Adam ; Römbke, Jörg

Abstract: A standardized bioassay previously developed with ivermectin for the yellow dung fly (Scathophagidae) and the face fly (Muscidae) was applied to test the response of 11 dung fly species to the presumably less toxic parasiticide moxidectin. The results were compared to existing data for the same species tested with ivermectin, albeit two new species (Scathophaga suilla and Musca domestica) were tested here with both the substances. Estimated lethal effect concentrations LC50 at which 50% of the flies died ranged more than tenfold from 0.012 mg moxidectin/kg fresh dung for Sepsis neocynipsea (Sepsidae) to 0.140 mg moxidectin/kg fresh dung for the house fly Musca domestica (Muscidae). In most of the species, we additionally revealed sub-lethal effects at lower moxidectin concentrations in terms of retarded growth and development and reduced body size. Mortality thresholds were about ten times higher for moxidectin than for ivermectin, hence moxidectin is indeed less toxic than ivermectin in absolute terms. Crucially, we obtained strong correlations among the 11 tested fly species in both lethal and sub-lethal responses to the two substances, such that species relatively sensitive to ivermectin were also relatively sensitive to moxidectin. Such correlations are expected if the two substances are structurally related and function in the same manner by disturbing ion channel transport. Methodologically speaking, all species used proved suitable for toxicological testing of parasiticides.

DOI: https://doi.org/10.1016/j.ecoenv.2012.10.030

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-76001
Journal Article
Accepted Version

Originally published at:
Blanckenhorn, Wolf U; Puniamoorthy, Nalini; Scheffczyk, Adam; Römbke, Jörg (2013). Evaluation of eco-toxicological effects of the parasiticide moxidectin in comparison to ivermectin in 11 species of dung flies. Ecotoxicology and Environmental Safety, 89:15-20.
DOI: https://doi.org/10.1016/j.ecoenv.2012.10.030

*Manuscript
Click here to view linked References

Toxicological Tests of the Livestock Parasiticide Moxidectin in Comparison to Ivermectin using Various Dung Flies

Wolf U. Blanckenhorn[a*], Nalini Puniamoorthy[a], Adam Scheffczyk[b], Jörg Römbke[b*]

a) Institut für Evolutionsbiologie & Umweltwissenschaften, Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

b) ECT Oekotoxikologie GmbH Böttgerstr. 2-14, D-65439 Flörsheim, Germany

*Author for correspondence
Dr. Wolf Blanckenhorn
Zoological Museum
Evolutionary Biology & Environmental Studies
University of Zurich-Irchel
34 (building)-J (floor) -98 (office)
Winterthurerstrasse 190
CH-8057 Zurich
Phone: +41 44 635.47.55
E-mail: wolf.blanckenhorn@ieu.uzh.ch

Running head: Dung fly tests with Moxidectin and Ivermectin

Word count: 3788 words; 4 Figures; 2 Tables

Abstract — A standardized bioassay previously developed with ivermectin for the yellow dung fly (Scathophagidae) and the face fly (Muscidae) was applied to test the response of 11 dung fly species to the presumably less toxic parasiticide moxidectin. The results were compared to existing data for the same species tested with ivermectin. Estimated lethal effect concentrations LC50 at which 50% of the flies died ranged more than tenfold from 0.012 mg moxidectin / kg fresh dung for *Sepsis neocynipsea* (Sepsidae) to 0.140 mg moxidectin / kg fresh dung for the house fly *Musca domestica* (Muscidae). In most species we additionally revealed sub-lethal effects at lower moxidectin concentrations in terms of retarded development and reduced body size. Mortality thresholds were about ten times higher for moxidectin than for ivermectin, hence moxidectin is indeed less toxic than ivermectin to the tested species. Crucially, we obtained strong correlations among the 11 tested fly species in both lethal and sub-lethal responses to the two substances, such that species relatively sensitive to ivermectin were also relatively sensitive to moxidectin. Such correlations are unsurprising because the two substances are structurally related, and function in the same manner by disturbing ion channel transport. Methodologically speaking, all species used proved suitable for the toxicological testing of parasiticides. Particularly interesting in this context would be comparisons in future studies with substances that feature entirely different modes of action. The existing OECD guideline fly test guideline could be modified in order to allow sepsid testing as well.

Keywords — Dung community, Insect, *Musca*, *Scathophaga*, Toxicological test, Sepsidae.

Introduction

Manufacturers of veterinary pharmaceuticals are required to conduct environmental risk assessment studies as part of the registration process to demonstrate the quality, safety and efficacy of any new products. The Veterinary International Co-operation on Harmonization (VICH) coordinates such registration regulations in various industrialized countries (VICH, 2004). Over the past decade, the SETAC (Society for Environmental Toxicology and Chemistry) advisory group DOTTS (Dung Organism Toxicity Testing Standardization) has been active in developing guidelines for testing for residues of veterinary pharmaceuticals excreted in the dung of treated livestock. Over the years, many laboratory tests and field studies have revealed that typically some proportion of dung dwellers, primarily beetles and flies, are negatively affected by such pharmaceutical residues (e.g. Wall and Strong, 1987), ultimately impeding their important ecosystem function of breaking down the dung (reviewed by Floate et al., 2005; Jochmann et al., 2011). Such unintended, non-target effects have raised concerns, to the extent that regulators now mandate standardized toxicological tests of dung dwelling organisms (VICH, 2004). As a result of the DOTTS activities, several studies appeared recently demonstrating the feasibility and reliability of such laboratory tests, using two flies (the yellow dung fly, *Scathophaga stercoraria* L. (Diptera: Scathophagidae), and the face fly *Musca autumnalis* L. (Diptera: Muscidae)) (Römbke et al., 2009, 2010)) as test species, and ivermectin, the oldest and still most commonly used parasiticide (Õmura, 2008), as reference substance. These fly tests have already been validated and standardized (OECD, 2008; OECD, 2005).

It should be obvious that no single test species can possibly capture, and hence typify, the diversity of sensitivities to any particular toxic substance. Moreover,

76 worldwide standards necessarily require use of several test species representing
77 different taxonomic groups (e.g. beetles vs. flies) and biogeographic regions of the
78 world (e.g. tropics vs. temperate zones.

79 It is equally clear that tests of one particular substance, such as e.g.
80 ivermectin, likely do not reveal the range of possible responses of any single species
81 to all available livestock medications, even if belonging to structurally related classes
82 (avermectins and milbemycins, in this case). In addition to testing several species, it
83 is therefore imperative to also test several different substances if we want to
84 understand the general impact such pharmaceutical residues have on natural
85 communities. If two pharmaceuticals are chemically related and belong to closely
86 related substance classes, as is the case for ivermectin and moxidectin (Fig. 1 + 2),
87 and if they even have the same functional mechanism – both substances disturb ion
88 transport through cell walls by binding to ion channels (Lumaret et al., 2012; Õmura,
89 2002) – a similar sensitivity of organisms to both pharmaceuticals might be expected.

90 **FIGURE 1 + 2**

91 Thus, we here test the response of a total of 11 fly species (Diptera), primarily
92 Sepsidae, that regularly breed in cattle dung to moxidectin, following the methods
93 and standards developed with ivermectin for yellow dung flies (Scathophagidae) and
94 face flies (Muscidae) (Römbke et al., 2009, 2010). Sepsid flies are distributed
95 worldwide (Blume, 1985; Pont and Meier, 2002), small, locally common, and easy to
96 rear in large groups (not unlike *Drosophila*) on cattle dung; they have short
97 generation times of ca. 2 weeks (e.g. Puniamoorthy et al., 2012). These properties
98 make them ideal test species for ecotoxicology. Moxidectin is chemically related to
99 ivermectin, but is believed to have relatively low toxicity (Doherty et al., 1994; Floate
100 et al. 2001; Lumaret et al., 2012; Õmura, 2002; Wardhaugh et al., 1996). In addition

to mortality effects in terms of the lethal effect concentration at which 50% of the individuals die (LC50), we assess non-lethal effects on growth rate, development time and body size, which previous studies revealed to be present, interesting and relevant for the natural situation (Jochmann et al., 2011; Römbke et al., 2009; but see Römbke et al., 2010). We compare the moxidectin responses to previously obtained (and largely published) data for the same fly species in response to ivermectin (Römbke et al., 2009; Römbke et al., 2010).

1. Material and methods

We generally followed the methods and standards specified in Römbke et al. (2009, 2010; OECD, 2008). All moxidectin tests were performed over several months in 2010 in our laboratory in Zürich. Dung was spiked with technical moxidectin (CAS No. 113507-06-5) supplied by Fort Dodge Animal Health (Monmouth Junction, NJ, USA). The substance was first dissolved in acetone to obtain the desired concentrations by serial dilution. The solution was then thoroughly mixed into cattle dung and kept overnight at room temperature to allow for evaporation of the solvent. A blank control, a solvent (acetone) control and a total of 9 concentrations ranging from 0.01 to 100 mg moxidectin / kg dung fresh weight were tested. As the dry matter content of the dung used was determined as 15.125%, these numbers were equivalent to 0.066 – 661.1 mg moxidectin / kg dung dry weight. No residue analysis was performed. Dung used in all tests was originally collected fresh from cattle in the field that had not been treated with parasiticides for at least three months, and had been kept frozen at -20 °C for at least 4 weeks before use.

All flies used were originally caught wild and propagated for multiple generations in our laboratory in Zürich. Most species stemmed from around Zürich,

126 Switzerland, except *Sepsis monostigma* (a subtropical species from China) and *S.*
127 *punctum* (collected in Berlin, Germany); *Musca autumnalis* was provided by the
128 Department of Entomology, University of Minnesota, Saint Paul, MN, USA.
129 The experimental units were plastic containers depending on the size of the
130 species filled with a set amount of test dung necessary for development of the larvae
131 (see Römbke et al., 2009, 2010). For the larger *Scathophaga* and *Musca* species we
132 used at least > 3 g per larva in a capped plastic vessel, and for the smaller *Sepsis*
133 species > 0.5 g dung per larva in 22 x 44 x 6 mm^3 plastic dishes positioned into a
134 capped 50 ml glass tube. There were 5 replicates per concentration. Typically 10 –
135 15 eggs (*Scathophaga* and *Musca spp.*) or newly hatched larvae (*Sepsis spp.*) from
136 several holding containers and/or mothers were counted into each experimental
137 vessel. The experimental containers were then incubated in a climate chamber at
138 21 °C. Emerging flies were collected daily from the containers.
139 The total number of adult flies (of both sexes) emerging from each container was
140 counted to compute the overall proportion of surviving flies. Sub-lethal effects were
141 additionally assessed by scoring the total egg-to-adult development times and body
142 sizes of all emerged flies. For the sepsids we used head width and for the larger
143 scathophagids and muscids fresh body mass. The sexes typically differ in these
144 parameters, so data for males and females were taken separately. Growth rates
145 could then be calculated simply as body size / development time.
146 As for binary data sigmoid relationships are expected, moxidectin
147 concentrations causing 50% mortality (LC50) were estimated using probit analysis of
148 logit-transformed emergence proportions against $\log_{10}$(moxidectin concentration),
149 separately for each species. Analogous linear regressions were employed to assess
150 the effect of $\log_{10}$ (moxidectin concentration) on development time, body size and

151 growth rate (untransformed raw values in all cases). (Acetone control was set to
152 0.005 moxidectin equivalents and blank control to 0.004 for purposes of analysis,
153 because otherwise all zero concentration values would have been excluded
154 automatically.) A test was considered valid only if larva-to-adult survival in the
155 combined blank and acetone control treatments exceeded 50%.

156 The ivermectin tests, conducted in our Zurich laboratory between 2008 and
157 2010, generally followed the same protocol as for moxidectin (Blanckenhorn et al.,
158 submitted). They were performed using technical ivermectin (CAS-No. 70288-86-7)
159 with a purity of 94% ivermectin B1a and 2.8% ivermectin B1b (supplied by Paul
160 Cooper, Merial, Atlanta, GA, USA). So far unpublished data on effects of ivermectin
161 on Swiss *Musca domestica* and *Scathophaga suilla* exactly followed the methodology
162 described in Römbke et al. (2010, 2009).

163

164 **2. Results**

165 Estimated Lethal Effect Concentrations LC50 (at which 50% of the flies died)
166 in terms of fresh dung and dry dung matter, with their (asymmetric) 95% confidence
167 intervals (CI), varied substantially among fly species in response to moxidectin
168 exposure (Table 1). *Sepsis neocynipsea* showed the lowest (0.012 mg / kg fresh
169 dung) and the house fly *Musca domestica* (0.140 mg / kg fresh dung) the highest
170 LC50 threshold, a difference of more than one order of magnitude. Fig. 3a shows
171 exemplary data for *Sepsis monostigma.*

172 **TABLE 1**

173 **FIGURE 3a-d**

174 Regarding the sub-lethal effects, 10 of 11 species showed a positive linear
175 relationship between development time and $\log_{10}$ (moxidectin concentration) (both

176 sexes combined; two-tailed binomial test: P = 0.011), of which 6 were individually
177 significant (Table 1). Exemplary data for *Sepsis monostigma* are displayed in Fig. 3b.
178 Similarly, all 11 species showed a negative linear relationship between body size and
179 $\log_{10}$ (moxidectin concentration), 8 being significant (Table 1; Fig. 3c). When
180 combining both effects in terms of growth rate (= body size / development time),
181 again all 11 species showed a negative relationship, 9 being significant (Table 1; Fig.
182 3d). Thus, a (sub-lethal) reduction of juvenile growth by moxidectin universally
183 occurred in all fly species.

184 The two new species tested with ivermectin differed considerably in sensitivity
185 from to their previously tested congeners (Römbke et al. 2010, 2009; Table 2). The
186 LC50 value in the test with *M. domestica* was about 5 times higher than that
187 determined for the standard species *M. autumnalis* (24.7 vs. 4.7 µg/kg). In contrast,
188 *Scathophaga suilla* was about 2.3 times more sensitive that the well-known test
189 species *S. stercoraria* (8.8 vs. 20.9 µg/kg).

190 **TABLE 2**

191 Comparing the moxidectin data obtained here with the corresponding data for
192 the same 11 species in response to ivermectin (published in Römbke et al. 2010,
193 2009; Blanckenhorn et al, submitted; and those two described in this paper), a
194 strongly positive correlation among species of r = 0.96 was obtained for the LC50
195 thresholds (Fig. 4a). Sub-lethal responses were correlated for development time (Fig.
196 4b) and growth rate (Fig. 4d) but not body size (Fig. 4c; note the missing of the
197 ivermectin estimates for *Musca autumnalis*: Table 2).

198 **FIGURE 4a-d**

199

3. Discussion

Standardized laboratory toxicological tests as developed for the yellow dung fly *Scathophaga stercoraria* and the face fly *Musca autumnalis* (Römbke et al., 2010, 2009) in response to ivermectin work well for other closely related *Scathophaga* and *Musca* species as well as various sepsid species. Sensitivities of a total of 11 fly species to moxidectin vary by about one order of magnitude, from LC50 = 0.012 mg / kg fresh dung for *Sepsis neocynipsea* to 0.140 mg / kg fresh dung for the house fly *Musca domestica*. Variation in ivermectin LC50 thresholds for these same 11 species was even greater, exceeding two orders of magnitude (Fig. 2a). Note that, in absolute terms, mortality thresholds are about 10-fold higher for moxidectin than for ivermectin (cf. Fig. 2a). (Doherty et al., 1994; Floate et al., 2002, 2001; Wardhaugh et al., 1996). Moxidectin concentrations used here are by no means exceptional in the field (e.g. in Japanese cattle dung the highest concentration three days after application was about 1 mg moxidectin / kg fresh dung (Iwasa et al., 2008)). This large inter-specific variation in responses suggests that any single test species cannot be representative in terms of assessing the toxicity of pharmaceutical residues for the dung community, making choice of appropriate test species difficult. We suggest using several test species, as done here, or, depending on the respective testing strategy (i.e. at which tier such a test is required) even the whole dung community (Floate et al., 2005; Jochmann et al., 2011).

As for ivermectin, our study also revealed sub-lethal effects at lower concentrations of moxidectin in terms of prolonged development and reduced growth, typically resulting in smaller final body sizes in all species (Table 1; Fig. 3; cf. Römbke et al., 2009, but see Römbke et al. 2010). Such sub-lethal effects in dung-breeding insects influence their performance in the natural habitat (Floate et al.,

225 2005; Jochmann et al., 2011), as smaller flies often have lower mating success in the
226 field (e.g. Jann et al., 2000), and longer development times can be detrimental in
227 time-limited situations when the winter is approaching or when the dung pat is drying
228 (e.g. Blanckenhorn, 1998). It is therefore sensible that the OECD Guideline for dung
229 flies (OECD, 2008) recommends measuring developmental time as well as
230 morphological traits such as body size in addition to mortality (LC50) effects. Such
231 measurements require little additional effort, yet can be sensitive indicators for the
232 presence of toxic effects of residues.

233 Our salient result is the strong correlation across the 11 tested dung fly
234 species in lethal and sub-lethal responses to moxidectin and ivermectin (Fig. 4). That
235 is, a species relatively sensitive to ivermectin is also relatively sensitive to
236 moxidectin. Somewhat less stringently, this extends to the sub-lethal responses in
237 terms of development and growth retardation, although not to body size reduction.
238 We expected such correlations because the two substances are structurally similar
239 (Fig. 1, 2), belonging to two related classes of parasiticides (avermectins and
240 milbemycins). Furthermore, both substances have a comparable mode-of-action
241 (Lumaret et al., 2012) in disturbing ion transport through cell walls by binding to ion
242 channels. Therefore correlated sensitivities of dung organisms to these two
243 substances, and probably also to other avermectins, are not surprising. Probably all
244 avermectins and milbemycins perturb molting by disturbing ion channel transport,
245 although this remains to be verified. According to the most recent phylogenetic
246 hypothesis largely supported by molecular data, the roundworms (Nematoda; in fact,
247 all the Nematoida, which additionally include the parasitic horsehair worms =
248 Nematomorpha) cluster together with the arthropods as Ecdysozoa (moulting
249 animals). The old classification of arthropods with the segmented worms (Annelida)

as Articulata (segmented animals) is now outdated. While avermectins were intended to primarily target parasitic Nematoida, they also disturb moulting of arthropods as a side effect. This also explains why flatworms (Platyzoa) and tapeworms (Cestoda), two other prominent parasitic worm groups, are perhaps not affected by avermectins: they do not belong to the Ecdysozoa and do not moult. In fact, one may argue that any pharmaceutical that kills parasites by perturbing the moulting process is too unspecific, as the by far largest group of higher animals, the arthropods, will be inadvertently affected, which include many beneficial insects such as dung decomposing flies and beetles. Most interesting in the context here would be comparisons with a parasiticide that features an entirely different mode of action.

In conclusion, at this point in time a number of dung fly species have been toxicologically tested with ivermectin using the same protocol (Römbke et al., 2010, 2009; Blanckenhorn et al., submitted). This study adds to the literature by testing the same dung flies also with moxidectin, in general finding responses well correlated with those for ivermectin. All fly species proved suitable for toxicological testing, which consequently can be considered established and practical, and might consequently be added to the relevant OECD guidelines.

Acknowledgements — We thank the German Umweltbundesamt (UBA), notably Nicole Adler, for funding this project, members of the DOTTS ring test group for input, and Fort Dodge Animal Health (Monmouth Junction, NJ, USA) and Merial (Atlanta, GA, USA) for providing the test substance.

5. References

Blanckenhorn, W.U., 1998. Adaptive phenotypic plasticity in growth rate and

diapause in the yellow dung fly. Evolution 52, 1394-1407.

Blanckenhorn, W.U., Puniamoorthy, N., Schäfer, M.A., Scheffczyk, A. & Römbke, J. : Standardized laboratory tests of the livestock antiparasiticide Ivermectin using various Sepsid dung fly species. Ecotox. Envir. Safety (submitted)

Blume, R.R., 1985. A checklist, distributional record, and annotated bibliography of the insects associated with bovine droppings of pastures in America north of Mexico. Southwest Entomol. Suppl. 9, 1-54.

Doherty, W.M., Stewart, N.P., Cobb, R.M., Keiran, P.J., 1994. In-vitro comparison of the larvicidal activity of moxidectin and abamectin against *Onthophagus* (Coleoptera, Scarabaeidae) and *Haematobia irritans* (Diptera, Muscidae). J. Austr. Entomol. Soc. 33, 71-74.

Floate, K.D., Wardhaugh, K.G., Boxall, A.B.A., Sherratt, T.N., 2005. Faecal residues of veterinary pharmaceuticals: non-target effects in the pasture environment. Ann. Rev. Entomol. 50, 153-179.

Floate, K.D., Colwell, D.D., Fox, A.S., 2002. Reductions of non-pest insects in dung of cattle treated with endectocides: a comparison of four products. Bull. Entomol. Res. 92, 471-481.

Floate, K.D., Spooner, R.W., Colwell, D.D., 2001. Larvicidal activity of endectocides against pest flies in the dung of treated cattle. Med. Vet. Entomol. 15, 117-120.

Iwasa, M., Suzuki, N., Maruyama, M., 2008. Effects of moxidectin on coprophagous insects in cattle dung pats. Japan. Appl. Entomol.Zool. 43, 271-280.

Jann, P., Blanckenhorn, W.U., Ward, P.I., 2000. Temporal and microspatial variation in the intensities of natural and sexual selection in the yellow dung fly *Scathophaga stercoraria*. J. Evol. Biol. 13, 927-938.

Jochmann, R., Blanckenhorn, W.U., Bussière, L., Eirkson, C.E., Jensen, J., Kryger,

300 U., Lahr, J., Lumaret, J.-P., Römbke, J., Wardhaugh, K.G., Floate, K.D., 2011.
301 How to test nontarget effects of veterinary pharmaceutical residues in livestock
302 dung in the field. Integr. Environ. Assess. Manag. 7, 287-296.
303 Lumaret, J.-P., Errouissi, F., Floate, K., Roembke, J. & Wardhaugh, K., 2012. A
304 review on the toxicity and non-target effects of macrocyclic lactones in the
305 terrestrial and aquatic environment. Curr. Pharm. Biotechnol. 13: 1004-1060.
306 OECD (Organisation for Economic Co-Operation and Development). 2008. OECD
307 guidelines for the testing of chemicals. Determination of Developmental Toxicity
308 of a Test chemical to Dipteran Dung Flies (*Scathophaga stercoraria* L.
309 (Scathophagidae), *Musca autumnalis* De Geer (Muscidae)). Final Draft. Paris,
310 France.
311 OECD (Organisation for Economic Co-Operation and Development). 2005. Guidance
312 document on the validation and international acceptance of new or updated test
313 methods for hazard assessment. OECD Environment, Health and Safety
314 Publications, Series on Testing and Assessment No. 34, 96 pp. Paris.
315 Õmura, S., 2008. Ivermectin: 25 years and still going strong. Int. J. Antimicrob. Ag.
316 31, 91–98.
317 Õmura, S., 2002. Mode of action of avermectin, in: Õmura, S., (Edt.), Macrolide
318 antibiotics: Chemistry, biology and practice. Academic Press, New York, pp. 571–
319 576.
320 Pont, A.C., Meier, R., 2002. The Sepsidae (Diptera) of Europe. Fauna Entomol.
321 Scand. 37, 1-221.
322 Puniamoorthy, N., Schäfer, M.A., Blanckenhorn, W.U., 2012. Sexual selection
323 accounts for geographic reversal of sexual size dimorphism in the dung fly,

Sepsis punctum (Diptera: Sepsidae). Evolution, doi:10.1111/j.1558-5646.2012.01599.x, in press.

Römbke, J., Barrett, K., Blanckenhorn, W.U., Hargreaves, T., Kadiri, N., Knäbe, S., Lehmhus, J., Lumaret, J.-P., Rosenkranz, B., Scheffczyk, A., Sekine, T., 2010. Results of an international ring test with the dung fly *Musca autumnalis* in support of a new OECD test guideline. Sci. Tot. Environ. 408, 4102-4106.

Römbke, J., Floate, K.D., Jochmann, R., Schäfer, M.A., Puniamoorthy, N., Knäbe, S., Lehmhus, J., Rosenkranz, B., Scheffczyk, A., Schmidt, T., Sharples, A., Blanckenhorn, W.U., 2009. Lethal and sublethal toxic effects of a test chemical (Ivermectin) on the yellow dung fly (*Scathophaga stercoraria*) based on a standardized international ring test. Environ. Toxicol. Chem. 28**,** 2117-2124.

VICH (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products). 2004. Environmental impact assessment for veterinary medicinal products – Phase II. Guidance. VICH GL 38, London, UK.

Wall, R., Strong, L., 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. Nature 327, 418-421.

Wardhaugh, K.G., Holter, P., Whitby, W.A., Shelley, K., 1996. Effects of drug residues in the faeces of cattle treated with injectable formulations of ivermectin and moxidectin on larvae of the bush fly, *Musca vetustissima* and the house fly, *Musca domestica*. Austr. Vet. J. 74, 370-374.

Figure Captions

Figure 1: Structural formulae of (a) ivermectin and (b) moxidectin.

Figure 2: Exemplary plots for the sub-tropical Chinese species *Sepsis latiforceps* (all ± SE): (a) proportion of adult flies (p) emerged for both sexes combined, and sex-specific (males denoted by squares and females by circles) (b) development times, (c) body size (head width), and (d) growth rates as a function of moxidectin concentration plus blank water (W) and acetone (A) controls. Concentrations > 3.16 mg moxidectin / kg fresh dung were tested but resulted in total mortality and are therefore not displayed.

Figure 3: Correlation between the responses to moxidectin and ivermectin for 11 dung fly species in (a) lethal threshold sensitivity (LC50), (b) development time, (c) body size, and (d) growth rate (cf. data in Table 1).

Table Caption

Table 1: Proportion of flies (p) emerged from the control treatments (water, acetone) for all 11 test species, plus the lethal concentration (LC50) of moxidectin (top) and ivermectin (bottom) at which 50% of the flies died, with their (asymmetric) 95% confidence limits in terms of fresh and dry dung. The last three columns give the correlation coefficient, for both sexes combined, between the life history trait and log10(moxidectin concentration). Significant correlations are in bold.

Table1

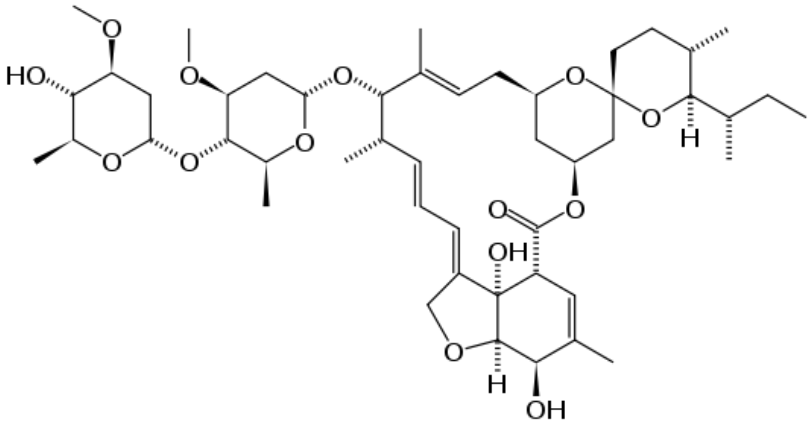
Table 1: Proportion of flies emerged from the control treatments (water, acetone) for all 11 test species, plus the lethal concentration (LC50) of moxidectin (top) and ivermectin (bottom) at which 5(

Moxidectin				**Wet dung**			**Dry dung**			***r***			
Family	Genus	Species	**p(emerged)**	**LC50 (µg / kg)**	**CI95%l**	**CI95%h**	**LC50 (mg / kg)**	**CI95%l**	**CI95%h**	**Development time**	**Body size**	**Growth rate**	
Sepsidae	*Sepsis*	*cynipsea*	0.794	**19.962**	14.532	27.296	**131.982**	96.076	180.472	0.286	**-0.324**	**-0.370**	
Sepsidae	*Sepsis*	*duplicata*	0.505	**15.000**	10.000	22.000	**99.174**	66.116	145.455	0.122	-0.143	-0.167	
Sepsidae	*Sepsis*	*fulgens*	0.762	**39.199**	24.691	61.520	**259.169**	163.246	406.741	**0.658**	**-0.564**	**-0.724**	
Sepsidae	*Sepsis*	*latiforceps*	0.808	**92.141**	52.253	162.419	**609.199**	345.472	1073.845	**0.461**	**-0.490**	**-0.532**	
Sepsidae	*Sepsis*	*neocynipsea*	0.690	**11.804**	7.253	18.501	**78.045**	47.951	122.318	0.262	**-0.674**	**-0.616**	
Sepsidae	*Sepsis*	*orthocnemis*	0.740	**24.062**	15.247	37.081	**159.085**	100.805	245.162	**0.548**	**-0.345**	**-0.586**	
Sepsidae	*Sepsis*	*punctum*	0.642	**26.159**	12.895	49.236	**172.950**	85.258	325.528	**0.671**	-0.161	**-0.533**	
Scathophagidae	*Scathophaga*	*stercoraria*	0.800	**121.000**	93.000	182.000	**800.000**	614.876	1203.306	**0.603**	**-0.411**	**-0.730**	
Scathophagidae	*Scathophaga*	*suilla*	0.852	**88.411**	59.568	130.868	**584.533**	393.838	865.244	-0.137	**-0.670**	**-0.646**	
Muscidae	*Musca*	*autumnalis*	0.630	**70.405**	26.719	165.909	**465.486**	176.651	1096.922	0.043	-0.188	-0.163	
Muscidae	*Musca*	*domestica*	0.536	**139.528**	19.757	834.195	**922.497**	130.624	5515.340	**0.380**	**-0.353**	**-0.379**	

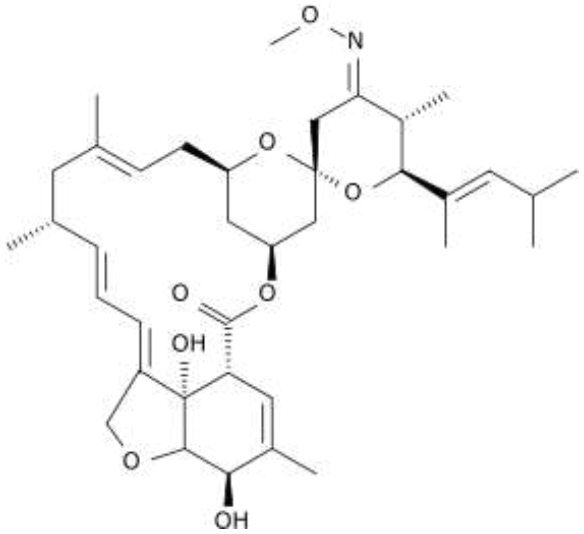
Ivermectin													
Family	Genus	Species											
Sepsidae	*Sepsis*	*cynipsea*	0.820	**0.491**	0.338	0.742	**3.500**	2.409	5.289	-0.075	-0.154	-0.136	Blanckenhorn et al., 2012
Sepsidae	*Sepsis*	*duplicata*	0.533	**0.090**	0.052	0.131	**0.641**	0.371	0.934	0.109	0.184	-0.013	Blanckenhorn et al., 2012
Sepsidae	*Sepsis*	*fulgens*	0.880	**5.567**	3.230	11.006	**39.679**	23.022	78.446	**0.588**	-0.214	**-0.528**	Blanckenhorn et al., 2012
Sepsidae	*Sepsis*	*latiforceps*	0.845	**11.438**	5.166	33.596	**81.525**	36.821	239.458	**0.343**	**-0.438**	**-0.459**	Blanckenhorn et al., 2012
Sepsidae	*Sepsis*	*neocynipsea*	0.703	**0.232**	0.190	0.286	**1.654**	1.354	2.038	0.173	0.008	-0.089	Blanckenhorn et al., 2012
Sepsidae	*Sepsis*	*orthocnemis*	0.737	**1.090**	0.694	1.739	**7.769**	4.947	12.395	**0.474**	-0.196	**-0.443**	Blanckenhorn et al., 2012
Sepsidae	*Sepsis*	*punctum*	0.794	**1.995**	1.216	3.505	**14.220**	8.667	24.982	**0.415**	**-0.492**	**-0.517**	Blanckenhorn et al., 2012
Scathophagidae	*Scathophaga*	*stercoraria*	0.744	**20.900**	10.900	27.500	**148.967**	77.691	196.009	**0.570**	**-0.372**	**-0.618**	Roembke et al., 2009
Scathophagidae	*Scathophaga*	*suilla*	0.714	**8.844**	5.297	15.782	**63.036**	37.755	112.488	0.201	**-0.429**	**-0.518**	this study
Muscidae	*Musca*	*autumnalis*	0.601	**4.650**	1.700	10.900	**33.143**	12.117	77.691	-0.075			Roembke et al., 2010
Muscidae	*Musca*	*domestica*	0.542	**24.719**	9.176	102.322	**176.187**	65.403	729.309	**0.646**	0.134	-0.034	this study

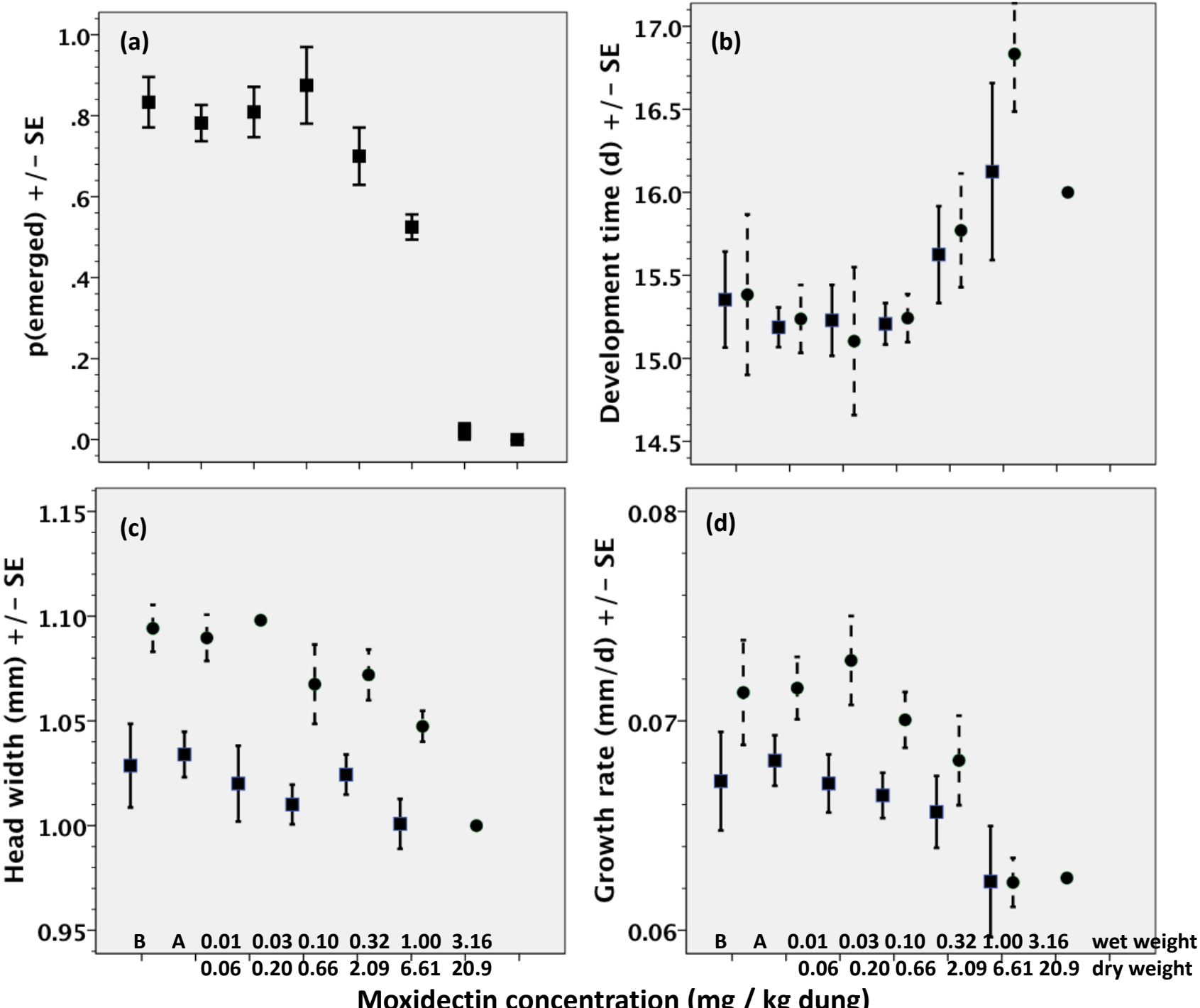
Figures1-3

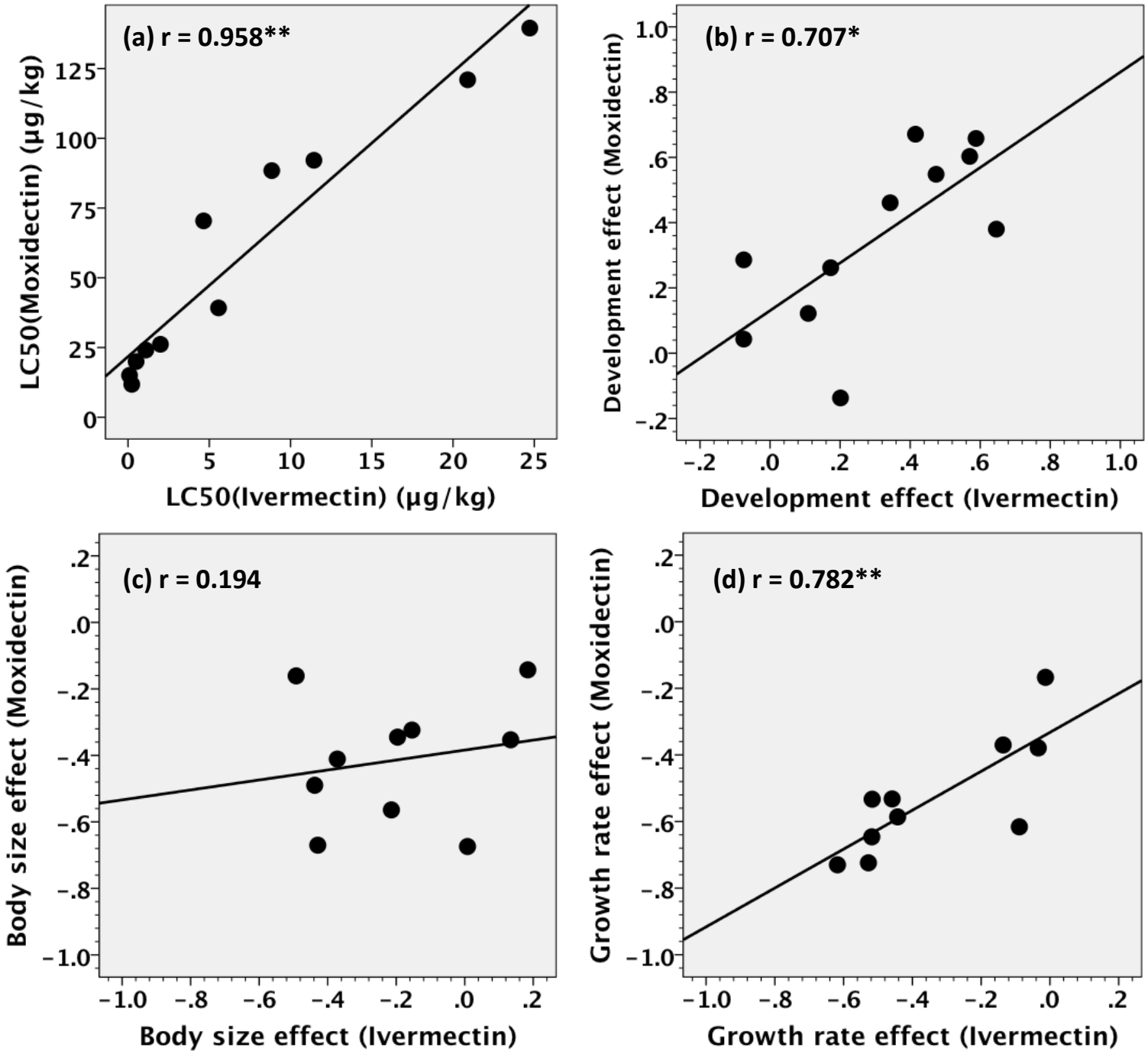
(a) Ivermectin



(b) Moxidectin




(a)
p(emerged) +/– SE
1.0
.8
.6
.4
.2
.0
(b)
Development time (d) +/– SE
17.0
16.5
16.0
15.5
15.0
14.5
(c)
Head width (mm) +/– SE
1.15
1.10
1.05
1.00
0.95
(d)
Growth rate (mm/d) +/– SE
0.08
0.07
0.06
B A 0.01 0.03 0.10 0.32 1.00 3.16 wet weight
0.06 0.20 0.66 2.09 6.61 20.9 dry weight
Moxidectin concentration (mg / kg dung)


(a) r = 0.958**
LC50(Moxidectin) (µg/kg)
LC50(Ivermectin) (µg/kg)
(b) r = 0.707*
Development effect (Moxidectin)
Development effect (Ivermectin)
(c) r = 0.194
Body size effect (Moxidectin)
Body size effect (Ivermectin)
(d) r = 0.782**
Growth rate effect (Moxidectin)
Growth rate effect (Ivermectin)